XANTHOPHYLLS AND CAROTENES OF DIATOMS, BROWN ALGAE, DINOFLAGELLATES, AND SEA-ANEMONES

HAROLD H. STRAIN, WINSTON M. MANNING AND GARRETT HARDIN

(*Carnegie Institution of Washington, Division of Plant Biology, Stanford University, California*)

Diatoms, dinoflagellates and brown algae, the principal autotrophic organisms of the sea, contain chlorophyll *a*, chlorophyll *c* (Strain and Manning, 1942a; Strain et al, 1943) and complex mixtures of yellow pigments. These yellow carotenoid pigments, which appear to play a role in photosynthesis (Dutton and Manning, 1941), have now been investigated by means of the highly selective chromatographic adsorption method. They have been compared with similar xanthophylls and carotenes from other sources such as leaves, flowers, and sea-anemones.

In the past seventy-five years there have been many investigations of the carotenoid pigments of diatoms and of brown algae. β-Carotene is the principal carotene and fucoxanthin is the principal xanthophyll (Palmer, 1922; Kylin, 1927; Walker, 1935; Heilbron, 1942). One or two additional fucoxanthin-like xanthophylls (Kylin, 1927; Montfort, 1940; Seybold and Egle, 1938; Seybold et al, 1941; Handke, 1941) have been regarded as native pigments (Kylin, 1927), as oxidation products of the principal fucoxanthin (Heilbron and Phipers, 1935; Kylin, 1939) or as interconvertible fucoxanthin isomers (Strain and Manning, 1942b). Other xanthophylls reported in brown algae are: fucoxanthophyll (Tswett, 1906a), xanthophyll or lutein (Palmer, 1922; Kylin, 1927; Heilbron, 1942; Montfort, 1940; Seybold and Egle, 1938; Seybold et al, 1941; Handke, 1941; Heilbron and Phipers, 1935; Kylin, 1939; Willstätter and Page, 1914; Carter et al, 1939; Pace, 1941), phyllorhodin or zeaxanthin (Kylin, 1939), cryptoxanthin and isolutein (Pace, 1941). Phylloxanthin, first reported both in brown algae and in leaves of higher plants, was found to be similar to the violaxanthin of pansy flowers (Kylin, 1927; 1939; Heilbron, Parry and Phipers, 1935). Zeaxanthin has also been regarded as a post-mortem product of brown algae (Heilbron and Phipers, 1935). Additional xanthophylls of diatoms have been reported as xanthophyll or lutein (Carter et al, 1939; Palmer, 1922; Kylin, 1927; Heilbron, 1942; Montfort, 1940; Seybold and Egle, 1938; Seybold et al, 1941; Handke, 1941; Pace, 1941), zeaxanthin, cryptoxanthin and isolutein (Pace, 1941).

There have been few investigations of the xanthophylls of dinoflagellates. The principal xanthophyll, called *peridinin* (Schütt, 1890; Kylin, 1927; Seybold et al, 1941), resembles the pigment sulcatoxanthin subsequently isolated from sea-anemones (Heilbron, Jackson and Jones, 1935). Another pigment of dinoflagellates is similar to the strongly adsorbed xanthophylls prepared from higher plants (Kylin, 1927; Strain, 1938a). The presence of lutein (xanthophyll) has also been reported (Seybold et al, 1941).

Experimental

Plant material. Brown algae were collected on the rocky reefs near Half Moon Bay, California. They were: *Fucus furcatus, Hesperophycus Harveyanus, Pelvetiopsis limitata, Cystoseira Osmundacea, Laminaria Andersonii, Pterygophora californica, Egregia Menziesii, Macrocystis integrifolia, Nereocystis pyrifera.*

Two colonial diatoms collected in quantity at the seashore were *Navicula torquatum* and *Isthmia nervosa.* Other diatoms, grown in pure culture or in unialgal culture, included *Nitzschia closterium, Nitzschia palea, Stephanopyxis turris* and *Thalassiosira gravida* (Strain and Manning, 1942a; Strain et al, 1943).

A fresh-water dinoflagellate, *Peridinium cinctum* (Strain et al, 1943), and a fresh-water yellow-green alga, *Tribonema bombycinum,* were found growing in a high state of purity as natural "blooms." A small, unicellular, brown colored alga was obtained in great concentration from the tentacles of the large, common, Pacific Coast sea-anemone *Bunodactis* (*Cribrina*) *xanthogrammica* (Strain et al, 1943). The amounts of algal material employed for preparation of the xanthophylls varied from 1 or 2 gm. of the centrifuged unicellular algae to 15 or 20 gm. of the brown algae. Only fresh, living algae were utilized.

Extraction of pigments. Absolute methanol containing about 0.5 per cent dimethylaniline was usually employed to extract the pigments from algae (Strain, 1938b; Strain and Manning, 1942a; Strain et al, 1943). Alteration of the chlorophyll to products that were difficult to remove from the xanthophylls was retarded by extraction of the plant material at room temperature with such a large quantity of methanol that the total amount of water present was not over 2 to 5 per cent.

Chromatographic behavior of pigments. Separation of the xanthophylls from the chlorophylls in extracts of many algae through use of saponification methods was not feasible, because some of the xanthophylls were altered by alkali. Partition of the pigments between methanol and petroleum ether failed to remove alcohol-soluble chlorophyll *c* from the xanthophylls. Modifications of the chromatographic adsorption method were finally utilized for separation of the chlorophylls and carotenes from the xanthophylls and for further resolution of the xanthophylls and carotenes.

No single adsorption procedure sufficed to separate all the algal pigments from one another. Many of the pigments were decomposed slowly by adsorption upon magnesium oxide. After partial separation of the pigments by adsorption upon columns of sugar, the incompletely separated, weakly adsorbed xanthophylls could be resolved further by adsorption upon columns of magnesia or by readsorption upon columns of sugar using mixtures of various solvents for development of the chromatograms. Under the different conditions, the relative positions occupied by the xanthophylls on the columns were often quite different (Strain, 1942a; 1942b; LeRosen, 1942).

Chromatographic preparation of pigments. Pigments contained in the methanol extracts of algae were transferred to petroleum ether by the addition of this solvent and aqueous salt solution. The green petroleum ether solutions were washed with water to remove residual methanol, concentrated to about 40 ml. at a temperature never above 20°, and then filtered through adsorption columns of dry powdered sugar (Strain, 1942a; 1942b; Strain et al, 1943), usually 3 or 4 cm. by 20 to 27 cm. In order to carry the last portions of the weakly adsorbed carotenes

below the other pigments, the columns were then washed with a little fresh petroleum ether, followed by petroleum ether containing 0.5 per cent *n*-propanol and 0.5 per cent dimethylaniline.

For spectroscopic examination of the carotene mixture small portions of the percolates were evaporated to dryness at reduced pressure. The residual carotenes were dissolved in 95 per cent ethanol, and the absorption spectra of the solutions were determined. For further identification of the carotenes, the bulk of the petroleum ether percolates were extracted several times with very dilute hydrochloric acid (0.5 per cent) which removed the dimethylaniline. The carotenes were then identified by adsorption upon a column prepared from a mixture of Micron Brand magnesium oxide No. 2641 and heat-treated diatomaceous earth (Filter Aid 501) (Strain, 1938a; 1942b).

Continued washing of the sugar columns with petroleum ether containing 0.5 per cent *n*-propanol caused the weakly adsorbed xanthophylls to separate from the chlorophyll *a* and from the strongly adsorbed xanthophylls and chlorophyll *c*. Resolution of the strongly adsorbed xanthophylls proceeded much more rapidly when petroleum ether with about 2 per cent *n*-propanol was subsequently used to develop the chromatogram. In order to accelerate the separation of the pigments and to avoid contamination of the resolved compounds with isomerization products that formed slowly, the weakly adsorbed and the strongly adsorbed xanthophylls were usually prepared rapidly on separate columns. For similar reasons, only freshly prepared extracts of the algae were utilized.

Petroleum ether solutions of several different alcohols were tested as solvents for the separation of algal pigments adsorbed upon columns of sugar. The resolving effect of aliphatic alcohols of various molecular weights was roughly equivalent to the amount of hydroxyl group that was present in the petroleum ether solution. Alcohols of higher molecular weight, such as amyl alcohol, produced slightly better separations of the pigments than alcohols of low molecular weight. At concentrations above 3 per cent, methanol tended to separate from the petroleum ether as a distinct phase in the adsorption columns. Alcohols of high molecular weight remained in solution, but they were difficult to remove from the petroleum ether by extraction with water and thus hindered further purification of the pigments by readsorption.

Pigments separated upon the adsorption columns were often contaminated by trailing portions of the substances which had preceded them (Strain, 1942a). All pigment preparations were purified by readsorption under various conditions until superposable spectral curves were obtained. The small quantities of the readsorbed pigments and the lability of many of them precluded the use of purification procedures involving crystallization.

Determination of properties of xanthophylls and carotenes. The most useful properties proved to be *partition between immiscible solvents* such as aqueous methanol and petroleum ether, *color reactions* produced by strong acids such as concentrated hydrochloric acid in the presence of ether, *comparative adsorbabilities* usually determined by adsorption upon Tswett columns of a mixture by the substances to be compared (Strain, 1942b), and *spectral absorption characteristics.*

Spectral absorption properties of the xanthophylls, determined with a photoelectric spectrophotometer (Smith, 1936), are plotted as the so-called characteristic absorption curves, the plot of log log (I_0/I) *versus* wave-length. Because of the

Color of adsorbed pigments	Name of pigments	Wave-lengths of absorption maxima (ethanol, mμ)	
Light green	Chlorophyll *c*		
Orange	Neofucoxanthin A	447	
Yellow-orange	Neofucoxanthin B	446	
Orange	Fucoxanthin	453	
Yellow	Diadinoxanthin	448	478
Yellow	Diatoxanthin	453	481
Green	Chlorophyll *a*		
Traces of yellow, green and gray	Xanthophylls Chlorophyll *a'* Pheophytin *a*		
Yellow	Carotenes		

FIGURE 1. Diatom pigments separated by adsorption upon a column of powdered sugar.

lability of the pigments, the small quantities obtained, and the presence of colorless concomitants, determination of the specific absorption coefficients was not feasible. Relative absorption values were always determined within a few hours after purification of the pigments by adsorption.

Results

Xanthophylls of diatoms. All the diatoms yielded the same series of xanthophyll pigments shown in Figure 1. However, different species showed significant variations in the relative amounts of the pigments.

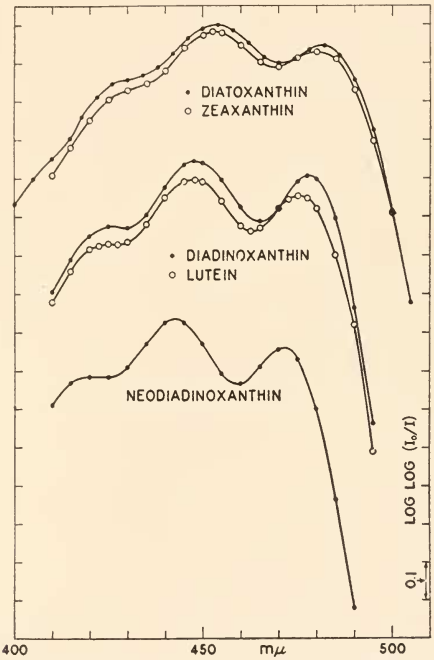


Figure 2. Characteristic spectral absorption curves of diatoxanthin, zeaxanthin, diadinoxanthin, luetin and neodiadinoxanthin. Solvent ethanol (95 per cent).

Diatoxanthin, a new xanthophyll, formed a paler but distinctly more orange band than the pigment adsorbed above it. Purified by readsorption, diatoxanthin yielded the spectral absorption curve shown with the similar zeaxanthin curve (Strain, 1938a) in Figure 2.

When dissolved in ether and treated with concentrated hydrochloric acid, diatoxanthin did not yield colored products. A mixture of diatoxanthin and zeaxan-

thin (the latter from the calyx of *Physalis alkekengi*) (Strain, 1938a) adsorbed from petroleum ether on a column of sugar and washed with petroleum ether containing one per cent methanol was resolved very slowly, zeaxanthin forming the lower band.

Diatoxanthin, dissolved in *n*-propanol and heated on a boiling water bath for one hour, underwent reversible isomerization, yielding some *neodiatoxanthin*. This second pigment was more strongly adsorbed and formed a yellower band, on a column of sugar, than the unchanged diatoxanthin (petroleum ether with 0.5 per cent *n*-propanol as wash liquid). Spectral absorption maxima of neodiatoxanthin were less pronounced and occurred at wave-lengths about 6 mμ shorter than those of diatoxanthin. By analogy with isomerization reactions of other carotenoid pigments (Strain, 1941; Polgár and Zechmeister, 1942), neodiatoxanthin probably represents a labile modification of the polyene system present in the molecule, diatoxanthin representing the stable or *trans* configuration.

In respect to color and position on the sugar columns, adsorbed neodiatoxanthin resembled the yellow band containing diadinoxanthin found next above the diatoxanthin when pigments of diatoms were adsorbed (see Fig. 1). However, diadinoxanthin and neodiatoxanthin did not yield identical spectral absorption curves. When a mixture of the two pigments was adsorbed on a column of sugar (petroleum ether plus 0.5 per cent *n*-propanol as wash liquid), neodiatoxanthin was adsorbed above diadinoxanthin.

Except for slight interconversion, neither diatoxanthin nor neodiatoxanthin was altered by a strong solution of potassium hydroxide in methanol. The behavior of these two pigments upon heating their solutions indicates that neither one is a labile isomer of zeaxanthin. Not more than traces of neodiatoxanthin or of zeaxanthin could have been present in the diatoms.

Diadinoxanthin (see Fig. 1) resembled lutein in respect to its spectral absorption (see Fig. 2) and color reactions. Readsorbed with lutein upon a column of sugar (petroleum ether with 0.5 per cent *n*-propanol as wash liquid), the diadinoxanthin moved through the column only about two-thirds as fast as the lutein.

As illustrated in Figure 1, the leading portion of the band of adsorbed diadinoxanthin became quite diffuse while the upper boundary of the band remained sharp and concentrated. This unique distribution of the pigment adsorbed on the column was observed after the diadinoxanthin had been purified in various ways. Spectroscopic properties of the pigment from the leading and trailing portions of the band were identical. Readsorption of each of these fractions upon fresh columns of sugar again yielded bands with diffuse leading portions.

In solution in hot *n*-propanol, diadinoxanthin underwent rapid, reversible isomerization yielding the similar, more adsorbed *neodiadinoxanthin*. Spectral absorption properties of the two pigments may be compared in Figure 2.

Fucoxanthin and its isomers (Strain and Manning, 1942b) contained in the extracts of diatoms moved slowly through the adsorption columns as indicated in Figure 1. Instead of the names suggested previously for the isomeric fucoxanthins (Kylin, 1927; 1939; Strain and Manning, 1942b), it is now proposed to call the principal isomer *fucoxanthin*, the other isomers *neofucoxanthin* A and *neofucoxanthin* B in conformity with the nomenclature of isomeric carotenoid pigments introduced by Zechmeister and his co-workers (Polgár and Zechmeister, 1942; and incl. refs.).

When the pigments were extracted quickly from diatoms and adsorbed on sugar from solution in petroleum ether containing 2 per cent *n*-propanol, the same mixture of pigments was always obtained. Adsorption of the principal fucoxanthin isomer under these same conditions yielded only traces of the other two compounds. This indicated that all three fucoxanthins may be normal constituents of the diatoms.

Interconversion of the three fucoxanthins proceeded rapidly in ethanol at 76° and slowly at 20°. Interconversion was accelerated by light and by substances upon which the pigments were strongly adsorbed, as for example by powdered glass or sugar when petroleum ether was used as solvent. This interconversion was also catalysed by iodine, but when bases such as pyridine and dimethylaniline were not added to neutralize traces of hydriodic acid that were formed (Strain, 1941), other pigments were produced. About 90 per cent of the equilibrium mixture, obtained by heating a solution of any of the isomers, was the stable fucoxanthin.

When dissolved in ether and treated with concentrated hydrochloric acid, each of the fucoxanthin isomers was converted into acid-soluble blue compounds. Treated with alcoholic potassium hydroxide the isomers formed pale yellow pigments that gave a blue color reaction with very dilute hydrochloric acid. The reaction with alkali proceeded in vacuum and in hydrogen as well as in air.

In order to obtain consistent spectral absorption curves for the isomeric fucoxanthins it was necessary to develop the chromatograms directly with petroleum ether containing 2 per cent *n*-propanol and 0.5 per cent dimethylaniline and to *work rapidly*. With these precautions, the same spectral curves were obtained whether the pigments were prepared from the diatoms or by interconversion from any of the other isomers (see Fig. 3). Noteworthy is the presence of only one definite spectral absorption maximum in the curve of each pigment. The spectral absorption curve of a petroleum ether solution of the principal fucoxanthin isomer, which is also shown in Figure 3, exhibits very distinct maxima, in sharp contrast to the curve for an ethanol solution. These spectral properties correspond to those of other carotenoid pigments that contain carbonyl groups in conjugation with the polyene structure in the molecule (Heilbron and Lythgoe, 1936).

These results on the xanthophylls of diatoms are contrary to the numerous reports (*loc. cit.*) that lutein is a constituent of these organisms. It is probable that diadinoxanthin may have been mistaken for lutein, and diatoxanthin may have been mistaken for zeaxanthin.

Carotenes of diatoms. Five of the six diatom species investigated contained principally β-carotene with only traces of other more adsorbed polyene hydrocarbons. One species, *Navicula torquatum*, contained the weakly adsorbed ε-carotene in addition to β-carotene (Strain and Manning, 1943a).

Effect of light of various spectral qualities on the formation of xanthophylls in diatoms. Pure cultures of *Nitzschia closterium* which were grown continuously in light from fluorescent "snow white" tubular lamps exhibited a constant ratio between the several xanthophyll pigments. When these cultures were placed in the red light from neon tubular lamps there was a gradual increase in the proportion of diadinoxanthin. After 20 days, when the culture had increased manyfold, the proportion of diadinoxanthin to other pigments was nearly twice as great as that in the cells grown in light from white fluorescent lamps. At this time the proportions between the other xanthophylls had changed very little.

These experiments demonstrated that the proportions of the carotenoid pigments of this photosynthetically active organism vary in response to changes in the environment. Whether this change results from variation in the rate of synthesis or degradation of the different pigments or whether it results from accelerated growth of a strain of the organism that contains different proportions of the xanthophylls is not yet known.

Xanthophylls of brown algae. Adsorption of the pigments of brown algae upon columns of sugar yielded a series of bands similar to those obtained from diatoms except that there was much less yellow pigment between the fucoxanthin and the chlorophyll *a*. Traces of two pigments, adsorbed as pale, narrow bands above

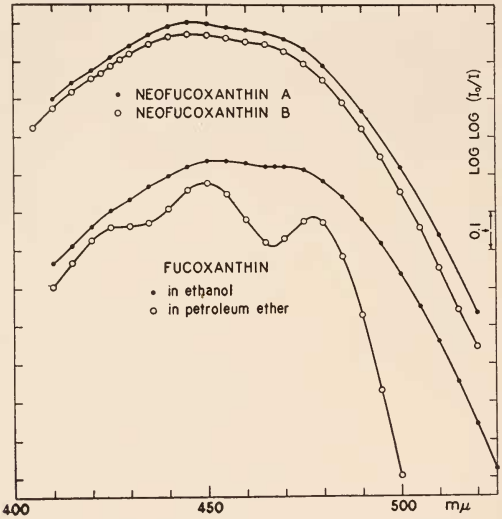


FIGURE 3. Characteristic spectral absorption curves of neofucoxanthin A, neofucoxanthin B and fucoxanthin dissolved in ethanol (95 per cent) and of fucoxanthin dissolved in petroleum ether.

chlorophyll *a*, appeared to be the diatoxanthin and the diadinoxanthin separated from the extracts of diatoms. The relative amounts of these two xanthophylls varied in different extracts of the same organism and in different species of brown algae. In view of the very small quantities of these two xanthophylls and their apparent absence in many species, it is uncertain whether the pigments came from the brown algae themselves or from diatoms that might have contaminated them.

When the pigments from brown algae were first adsorbed, a distinct yellow band usually appeared just below the orange fucoxanthin band. As the column was developed with petroleum ether containing propanol, this yellow band was often over-run by the orange fucoxanthin band. Under these conditions, the yellow pig-

ment, which exhibited spectral properties similar to those of violaxanthin, was observed again between the fucoxanthin and the neofucoxanthin B. If, however, the adsorbed pigments were washed with petroleum ether containing about 5 per cent acetone, the yellow band continued to advance ahead of the fucoxanthin, occasionally separating into two contiguous yellow bands. Pigment from the lower of these two yellow bands resembled the violaxanthin of leaves (Strain, 1938a), as is shown by the spectral absorption curves in Figure 4. Pigment from the upper yellow band exhibited a spectral absorption curve which (when corrected for absorption due to remaining violaxanthin) resembled the spectral curve of the flavoxanthin of leaves

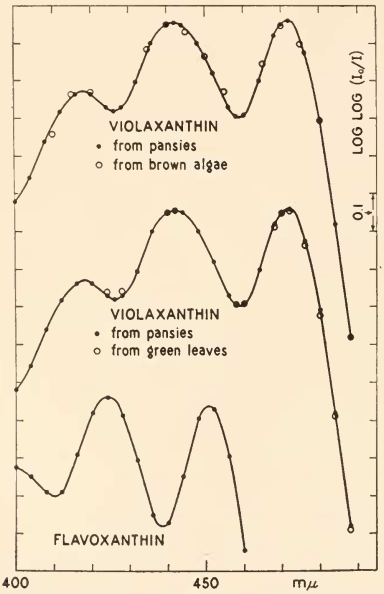


FIGURE 4. Characteristic spectral absorption curves of flavoxanthin and of violaxanthin from pansies, leaves and brown algae. Solvent, ethanol (95 per cent).

(Strain, 1938a). This flavoxanthin-like xanthophyll occurred in such small quantities that it could not be compared in other respects with the similar pigment of leaves.

The violaxanthin-like pigment from brown algae was found to be spectroscopically identical with violaxanthin prepared from leaves and from pansy flowers as described below (Fig. 4). Violaxanthin preparations from the several sources were also chromatographically identical. Mixtures of these preparations were inseparable by adsorption upon columns of magnesia (petroleum ether with 25 per cent acetone as wash liquid) or by adsorption upon columns of sugar (petroleum ether with 0.5 per cent *n*-propanol as wash liquid).

Changes in the relative positions of violaxanthin and of fucoxanthin, first observed when extracts of brown algae were adsorbed upon columns of sugar with different solvents as wash liquids, have been obtained with highly purified preparations of these two xanthophylls. With petroleum ether or with petroleum ether containing 5 per cent acetone as solvent, violaxanthin was adsorbed below fucoxanthin. With petroleum ether containing 0.5 per cent *n*-propanol as solvent, violaxanthin was adsorbed above fucoxanthin. Separated below fucoxanthin by adsorption from solution in petroleum ether containing acetone, violaxanthin gradually became adsorbed above the fucoxanthin when the column was washed with petroleum ether containing *n*-propanol.

Fucoxanthin and its isomers separated from the brown algae were identical with those separated from the diatoms as indicated by identical spectral absorption curves, adsorbability, and color reactions. Pigments that were extracted rapidly from fresh algal material and adsorbed from petroleum ether containing 2 per cent *n*-propanol yielded all three isomers; hence, these three pigments probably represent normal constituents of brown algae as well as of diatoms.

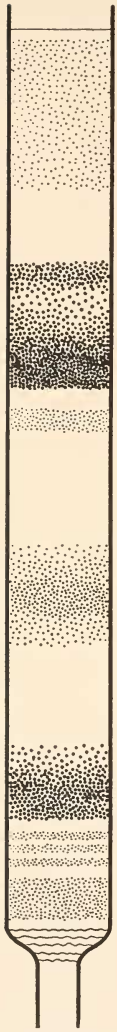
When the pigments of brown algae were adsorbed from solution in petroleum ether containing 2 per cent *n*-propanol, traces of xanthophylls with absorption spectra similar to that of neoxanthin from leaves (Strain, 1938a) were observed below and just above the fucoxanthin. Because of their weak adsorbability, neither of these pigments could have been neoxanthin. Their relations to other xanthophylls of similar spectra (Fig. 6) were not established. Traces of a xanthophyll with spectral properties similar to those of dinoxanthin (*vide infra*) were also observed on the column below the adsorbed fucoxanthin.

Carotenes separated from the brown algae were composed principally of the *beta* isomer with traces of more adsorbed carotenes. Neither α-carotene nor ϵ-carotene was observed.

These results on the carotenoid pigments of brown algae support the early observation of Tswett that fucoxanthophyll, the xanthophyll of these organisms, in addition to fucoxanthin, is different from xanthophyll (lutein) of leaves. They confirm the statement of Kylin (1927; 1939) that violaxanthin (phylloxanthin) is present in brown algae. They are in disagreement with the claims of Kylin and of many others, that lutein and zeaxanthin occur in many species of brown algae (some of which belong to the same genera as several of the species examined by us). In certain species of Laminaria and of Fucus, Heilbron and co-workers (Heilbron, 1942; Heilbron and Phipers, 1935; Carter et al, 1939) could not detect any xanthophylls other than fucoxanthin.

Our observations both on diatoms and on brown algae support Kylin's original statement (1927) and other recent claims (Montfort, 1940; Seybold and Egle, 1938; Strain and Manning, 1942b) that the several fucoxanthins are normal constituents of the plant cells. They are at variance with Heilbron and Phipers' contention that the additional fucoxanthin they observed is an oxidation product of the principal fucoxanthin. All these conclusions are difficult to reconcile with Kylin's latest statement (1939) that the pigment he regarded as fucoxanthin B may also have been an oxidation product. Zeaxanthin, reported as a normal constituent of brown algae by Kylin and by Pace and as a post-mortem product by Heilbron and Phipers, was not detected in our experiments.

Kylin (1939) has asserted that the violaxanthin of brown algae and of leaves



Color of adsorbed pigments	Name of pigments	Wave-lengths of absorption maxima (ethanol, mμ)	
Light green	Chlorophyll *c*		
Red-orange	Neoperidinin	464	
Red-orange	Peridinin	475	
Yellow	Neodinoxanthin	438	466
	Neodiadinoxanthin	442	471
Yellow	Dinoxanthin	441.5	471
	Diadinoxanthin	448	478
Green	Chlorophyll *a*		
Traces of yellow, green and gray	Xanthophylls Chlorophyll *a'* Pheophytin *a*		
Yellow	Carotenes		

FIGURE 5. Pigments of *Peridinium cinctum* (and of an alga from a sea-anemone) separated by adsorption upon a column of powdered sugar.

is identical with Tswett's (1906b) β-xanthophyll, the only carotenoid observed above chlorophyll *b* when leaf pigments were adsorbed upon columns of precipitated chalk with carbon disulfide as wash liquid. Repetition of Tswett's experiment using extracts of sunflower leaves has revealed that neoxanthin (Strain, 1938a) with traces of other xanthophylls forms the topmost yellow band. On columns of sugar with petroleum ether containing 0.5 per cent *n*-propanol as wash liquid, neoxanthin also formed the topmost band. Only after prolonged development of the chromatogram did violaxanthin appear above the band of adsorbed chlorophyll *b*. Under these conditions traces of a flavoxanthin-like xanthophyll appeared between the neoxanthin and the violaxanthin. This indicates that Tswett's β-xanthophyll may have been a mixture containing principally neoxanthin.

Xanthophylls of a dinoflagellate. Pigments extracted from the dinoflagellate *Peridinium cinctum* and adsorbed on a column of sugar yielded the series of bands shown in Figure 5. The yellow band which occurred next above the chlorophyll *a* proved to be a mixture of at least two pigments which were separated from each other by readsorption upon a column of sugar (petroleum ether with 5 per cent acetone or 0.5 per cent *n*-propanol as wash liquid). Diadinoxanthin, which formed the lower band, was spectroscopically and chromatographically identical with this pigment prepared from diatoms.

Dinoxanthin, separated from the diadinoxanthin by readsorption of the mixture from the original column, exhibited spectral absorption properties similar to those of violaxanthin and of taraxanthin (Fig. 6). In spite of the similarity in their spectra, these three pigments are distinct substances as demonstrated by their different adsorbabilities and their color reactions with acid. When the pigments were adsorped on sugar from solution in petroleum ether containing one per cent *n*-propanol, the adsorption order was: violaxanthin (topmost), dinoxanthin, and taraxanthin. On magnesia with petroleum ether containing 25 per cent acetone as solvent, the adsorption order was: dinoxanthin, violaxanthin and taraxanthin. Dissolved in ether and treated with concentrated hydrochloric acid, dinoxanthin and taraxanthin yielded only a trace of blue color in the acid layer whereas violaxanthin yielded a stable, deep blue color in the acid. Prior treatment of dinoxanthin with alcoholic potassium hydroxide did not affect its reaction with concentrated hydrochloric acid. None of these pigments was interconvertible by the action of heat on its solution.

There were indications that traces of a flavoxanthin-like xanthophyll occurred in admixture with the diadinoxanthin and the dinoxanthin. The quantities present were insufficient to permit definite identification.

As illustrated in Figure 5, there was always a small yellow band just below the principal red-orange band obtained by adsorption of the pigments of the dinoflagellates. Readsorption of the pigment from this band on a fresh column yielded neodiadinoxanthin as the lower band and an isomer of dinoxanthin as the upper band. In Figure 6, a spectral absorption curve of this second pigment, *neodinoxanthin,* may be compared with the curve of the more stable interconvertible dinoxanthin. Color and solubility reactions of the two pigments were similar.

In all the experiments, even though rapid and mild conditions of extraction and adsorption were employed, both neodinoxanthin and neodiadinoxanthin were observed in the extracts of the dinoflagellates. Because of the ease and rapidity with

which these two isomers are formed, it is not certain that they are normal constituents of *Peridinium*.

Peridinin, obtained from the principal red-orange band (illustrated in Fig. 5), exhibited some noteworthy properties. So far as can be ascertained, this pigment

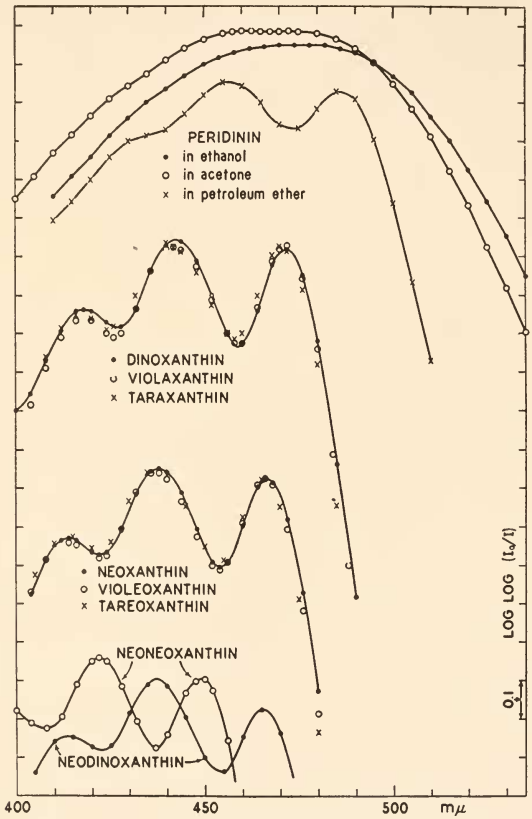


FIGURE 6. Characteristic spectral absorption curves of peridinin, dissolved in various solvents, and of dinoxanthin, violaxanthin, taraxanthin, neoxanthin, violeoxanthin, tareoxanthin, neodinoxanthin and neoneoxanthin dissolved in ethanol (95 per cent).

is the one observed in several species of *Peridinium* by Schütt (1890), by Kylin (1927), and by Seybold, Egle and Hülsbruch (1941). In solubility, peridinin resembled fucoxanthin, being extremely soluble in alcohols and but slightly soluble in petroleum ether. On sugar, it was much more adsorbed than fucoxanthin from

which it could be separated readily by development of the chromatogram with petroleum ether containing 2 or 3 per cent *n*-propanol. Peridinin was decomposed by alkalies, yielding paler pigments that did not give blue products when dissolved in ether and treated with concentrated hydrochloric acid. Peridinin itself, in contrast to fucoxanthin, did not yield a blue color with concentrated hydrochloric acid. This behavior of peridinin, like that of diadinoxanthin, is an exception to the rule that strongly adsorbed, alcohol-soluble xanthophylls yield a blue color with concentrated hydrochloric acid.

When dissolved in chloroform and treated with a solution of antimony trichloride in chloroform, peridinin formed a purple-orange solution that faded slowly to a lighter orange. Dissolved in chloroform and treated with concentrated sulfuric acid, peridinin caused the acid layer to turn deep blue. Crystals of peridinin, which formed readily when the solutions were concentrated, were turned deep blue by concentrated sulfuric acid.

Dissolved in methanol or in ethanol, peridinin exhibited strong absorption of light ranging from the violet to the yellow region of the spectrum. The spectral curve of alcohol and of acetone solutions showed a single broad absorption maximum. On the other hand, a solution of peridinin in petroleum ether exhibited two pronounced absorption maxima (see Fig. 6). Addition of as little as 2.5 per cent ethanol to a petroleum ether solution of peridinin caused a marked broadening of the absorption maxima. The petroleum ether solutions were quite yellow whereas the alcohol solutions were distinctly orange-red. By analogy with spectra of other carotenoid pigments (Heilbron and Lythgoe, 1936), these spectral properties indicate that the peridinin molecule contains at least one carbonyl group in conjugation with the polyene system.

Solutions of peridinin in carbon disulfide were pink-orange to red-orange in color. The peridinin in these solutions was very strongly adsorbed on sugar yielding a red adsorbate.

A dilute, yellow solution of peridinin in petroleum ether turned brick red when shaken with distilled water, and the pigment collected at the interface. This effect is similar to the color change observed when the peridinin is adsorbed on solids. Upon adsorption on solids or at a liquid-liquid interface, fucoxanthin also behaved similarly, but neoxanthin, which was more adsorbed than peridinin or fucoxanthin (see below), retained its characteristic yellow shade.

Peridinin, like other more typical xanthophylls, was moderately resistant to oxidation by atmospheric oxygen. Solutions of this xanthophyll in ethanol or in petroleum ether stored in open vessels in the dark were not bleached perceptibly in 2 or 3 months. In diffuse light from north windows, the solutions became colorless in a few weeks. In direct sunlight the solutions were decolorized in a few days.

When solutions of peridinin in *n*-propanol were heated on a boiling water bath, the pigment was isomerized rapidly. Products obtained by heating the solution for 2 hours were transferred to petroleum ether and adsorbed on a column of sugar using petroleum ether with 3 per cent *n*-propanol for development of the chromatogram. This resulted in the separation of a pale, diffuse red-orange band containing *neoperidinin* above the band containing the unchanged peridinin. Faint traces of other red-orange bands were adsorbed above the neoperidinin and very small quantities of still another pigment were adsorbed below the peridinin. In ethanol, all these pigments exhibited spectral absorption curves similar to that of peridinin but

their absorption maxima occurred at shorter wave-lengths. Traces of all these pigments were often observed when extracts of Peridinium were adsorbed upon columns of powdered sugar.

The carotene mixture extracted from *Peridinium cinctum* was composed almost entirely of β-carotene. The quantity of carotene present in the fresh centrifuged cells was greater than that obtained from the diatoms.

Xanthophylls of an alga from a sea-anemone. Algae squeezed from the tentacles of the sea-anemone, *Bunodactis xanthogrammica,* yielded the same series of pigments obtained from the dinoflagellates and tabulated in Figure 5. These pigments are constituents of the alga, not of the anemone.

Color reactions already described for peridinin from Peridinium were identical with those described for sulcatoxanthin from the sea-anemone *Anemonia sulcata* (Heilbron, Jackson and Jones, 1935). The absorption curve of peridinin dissolved in carbon disulfide exhibited two well-defined absorption maxima at 483 and 516 mμ, in good agreement with the values of 482 and 516 mμ reported for sulcatoxanthin. On the basis of available evidence, it appears that sulcatoxanthin is none other than the xanthophyll peridinin. As we have not been able to obtain the anemone *Anemonia sulcata* for analysis of its pigments, it is impossible to say whether the peridinin originates in the tissue of that animal or in the cells of the algae that are known to inhabit it (Fulton, 1922).

The identity of the pigments obtained from the symbiotic alga of Bunodactis and from the dinoflagellate suggests that both organisms may belong to the same or to related plant groups. From a microscopical examination of the symbiotic alga, Dr. H. W. Graham of Mills College concluded that this organism is not a dinoflagellate. It has been suggested that "zooxanthellae," the symbiotic algae of many sea anemones, may belong to another class of organisms, the cryptomonads (Fulton, 1922). If this is true, the autotrophic, free-living cryptomonads may also contain the same pigments found in the dinoflagellates.

Pigments of a yellow-green alga. The xanthophylls of *Tribonema bombycinum* proved to be different from those of all the other algae that we have investigated. Neither lutein nor any other common xanthophyll was observed. The several xanthophylls that were present formed pale yellow bands on the sugar columns, but these pigments have not been definitely identified. The carotene consisted almost entirely of the *beta* isomer. Tribonema did not contain chlorophylls *b, c,* or *d*.

Xanthophylls of dandelion flowers. For comparison with dinoxanthin, taraxanthin was prepared from flowers of the dandelion *Taraxacum officinalis* (Kuhn and Lederer, 1931). To this end, about 10 gms. of fresh dandelion flowers from which the stems and sepals had been cut were extracted with absolute ethanol; the extract was treated with an excess of potassium hydroxide; and after completion of the saponification, the xanthophylls were transferred to petroleum ether and adsorbed upon a column (3.7 by 22 cm.) of magnesia and siliceous earth (1:1). The chromatogram was then developed with petroleum ether containing 25 per cent acetone. Taraxanthin proved to be the principal, least adsorbed xanthophyll. After purification by readsorption it yielded the spectral absorption values shown in Figure 6.

Above the taraxanthin there were two or three adjoining bands one of which contained lutein. Above this group of bands, there appeared a yellow band which

yielded a hitherto undescribed pigment. This new xanthophyll, for which we propose the name *tareoxanthin,* exhibited a spectral curve almost identical in shape with that of neoxanthin from leaves (see Fig. 6) (Strain, 1938a).

Just above the band containing the tareoxanthin on the column with the adsorbed dandelion pigments there appeared a lemon yellow band. Xanthophyll eluted from this band exhibited a spectral absorption curve almost identical with that reported from flavoxanthin in ethanol (Strain, 1938a). Unlike flavoxanthin from other sources (Strain, 1938a; Kuhn and Brockmann, 1932), this xanthophyll from dandelions did not yield a blue product when dissolved in ether and treated with concentrated hydrochloric acid. This fact suggests that the "flavoxanthin" isolated from dandelion flowers by Karrer and Rutschmann (1942a) may not have been identical with flavoxanthin from other plants.

The mixture of dandelion xanthophylls was also resolvable by adsorption upon columns of sugar when petroleum ether containing one per cent *n*-propanol was used as solvent. Under these conditions the taraxanthin was adsorbed above the lutein.

Xanthophylls of pansy flowers. Violaxanthin, for comparison with the xanthophylls of algae, was prepared from the yellow flowers of *Viola tricolor* (Kuhn and Winterstein, 1931). Xanthophyll esters in the extracts of the flowers were saponified, and the free xanthophylls were crystallized from petroleum ether and aqueous alcohol. After recrystallization from petroleum ether and aqueous alcohol, the violaxanthin was purified further by adsorption on columns of magnesia and of sugar.

When the xanthophylls in the mother liquors from the violaxanthin preparation were adsorbed on magnesia or on sugar, relatively large quantities of a strongly adsorbed xanthophyll were observed (Karrer and Rutschmann, 1942b). This pigment, for which the name *violeoxanthin* is proposed, exhibited a spectral absorption curve almost identical with those of neoxanthin and of tareoxanthin (Fig. 6).

In spite of the similarity between the spectral absorption curves of neoxanthin, tareoxanthin, and violeoxanthin, these three pigments were readily separable by adsorption. Upon columns of magnesia with petroleum ether containing 25 per cent acetone as solvent or upon columns of sugar with petroleum ether containing one per cent *n*-propanol as solvent, neoxanthin formed the uppermost band, violeoxanthin the middle band, and tareoxanthin the lowest band. Dissolved in ether and treated with concentrated hydrochloric acid, violeoxanthin yielded an intensely blue, acid-soluble product whereas neoxanthin yielded no color, and the tareoxanthin yielded only traces of blue in the acid.

Violaxanthin from leaves. Violaxanthin was rapidly preparable from leaves by adsorption of a petroleum ether solution of the extracted pigments upon a column of magnesia and siliceous earth followed by development of the chromatogram with petroleum ether containing 25 per cent anhydrous acetone. Under these conditions, the violaxanthin was adsorbed far below the chlorophylls and below lutein. As a consequence it was not contaminated with other leaf pigments and was nearly spectroscopically homogeneous after a single adsorption.

Neoxanthin from leaves. When the pigments extracted from leaves were dissolved in petroleum ether and adsorbed upon a column of heat-treated siliceous earth (Filter Aid 501), the chlorophylls and xanthophylls were more adsorbed than upon a column of sugar. As the adsorbed pigments were washed with petroleum

ether containing 0.5 per cent *n*-propanol, neoxanthin formed the uppermost yellow band. Owing to the high filtration rate of the columns, considerable neoxanthin could be separated in a few minutes. Purified by readsorption upon fresh columns, the neoxanthin exhibited a spectral absorption curve identical with that of material prepared by adsorption upon magnesia from solution in dichloroethane (Strain, 1938a). It did not exhibit a blue color when dissolved in ethyl ether and treated with concentrated hydrochloric acid.

Dissolved in *n*-propanol and heated on a boiling water bath for several hours, neoxanthin was converted into two isomers that were more adsorbed than the unchanged neoxanthin. The most adsorbed isomer, *neoneoxanthin* A, exhibited spectral absorption maxima at wave-lengths almost identical with those reported for the absorption maxima of flavoxanthin, but its absorption maxima were not so pronounced (Fig. 6). These isomerization experiments indicate that neoxanthin contains the stable form of the chromophoric polyene group.

Both violaxanthin and neoxanthin were obtained from leaves that had been extracted at room temperature, from those that had been killed by boiling and from those that had been extracted with alcohol containing much dimethylaniline. These xanthophylls were also obtained when the leaf extracts were adsorbed directly on magnesia, on sugar or on siliceous earth or when they were adsorbed after treatment with alcoholic potassium hydroxide. They were not formed from lutein when this xanthophyll was exposed to the conditions utilized for the extraction and separation of the leaf pigments. These observations provide further indication that neoxanthin and violaxanthin are normal constituents of green leaves.

Relative adsorbabilities of xanthophylls. In addition to those reversals in the relative positions of adsorbed xanthophylls already reported, dinoxanthin was found to be adsorbed above diadinoxanthin in columns of sugar (solvents: petroleum ether containing 0.5 per cent *n*-propanol, 5 per cent acetone or 8 per cent methylisobutylketone). But, on magnesia with acetone or methylisobutylketone as solvents, dinoxanthin was adsorbed below diadinoxanthin.

A mixture of lutein and zeaxanthin was not readily separable on a column of sugar when petroleum ether with 5 per cent acetone was used as solvent. Adsorbed upon magnesia from solution in petroleum ether containing 25 per cent acetone, zeaxanthin was adsorbed far above lutein.

TABLE I

Relative positions of xanthophylls on columns of magnesia and of sugar with various solvents

	Magnesia	Magnesia	Sugar	Sugar
Solvent.....	Dichloro-ethane	Petroleum ether + 25% acetone	Petroleum ether + 25% acetone	Petroleum ether + 1% *n*-propanol
Xanthophylls...	Neoxanthin Violaxanthin Zeaxanthin Lutein	[Neoxanthin * Zeaxanthin] Lutein Violaxanthin	Neoxanthin Violaxanthin [Zeaxanthin * Lutein]	Neoxanthin Violaxanthin [Zeaxanthin * Lutein]

* Brackets indicate pigments that did not separate into bands.

Relative positions of the xanthophylls in the columns have been found to vary with changes in either the solvent or the adsorbent. This is illustrated by the results summarized in Table I.

Because knowledge of the relative positions of known xanthophylls in the adsorption columns aids in the preparation and identification of these pigments from various sources, the adsorption orders of many of the xanthophylls described in this paper are listed in Table II. For these comparisons, mixtures of two or three of

TABLE II

Relative positions of xanthophylls on columns of magnesia and of sugar

	Magnesia	Sugar
Solvent	Petroleum ether + 25% Acetone	Petroleum ether + 0.5–1% *n*-propanol
Xanthophylls	Neoneoxanthin A Neoneoxanthin B [Neoxanthin * [Zeaxanthin Fucoxanthin Peridinin [Violeoxanthin * [Lutein Dinoxanthin [Tareoxanthin * [Violaxanthin Taraxanthin Cryptoxanthin	Neoxanthin Peridinin Neofucoxanthin A Neofucoxanthin B Violeoxanthin Violaxanthin Fucoxanthin Tareoxanthin Taraxanthin [Zeaxanthin * [Lutein Cryptoxanthin

* Brackets indicate pigments that did not separate into discrete bands.

the xanthophylls, dissolved in petroleum ether, were adsorbed, and the chromatograms were then developed with petroleum ether containing the polar solvent.

Changes in the relative adsorbabilities of the xanthophylls with variations of the solvent are apparently related to the solubilities of the pigments as well as to effects of the solvents upon the chromophoric groups (see Fig. 6). For example, peridinin and fucoxanthin, which are adsorbed below zeaxanthin when acetone is used as solvent, are more soluble in this ketone than is zeaxanthin.

DISCUSSION

Algal pigments. A number of new xanthophylls have been isolated from the algae investigated. Additional pigments might be obtained by the use of larger quantities of plant material followed by readsorption of the minor bands on smaller columns. Utilization of additional refinements in technique may result in further resolution of the pigments described in this paper, especially the isomerization products of the stable xanthophylls. However, it seems improbable that such pigments would represent a considerable proportion of the total xanthophylls.

The relatively great adsorbability of the algal xanthophylls indicates that these hydroxy compounds do not occur in the form of esters. In this respect, xanthophylls of algae resemble those of the chloroplasts of higher plants (Strain, 1938a).

Occurrence of the unesterified xanthophylls in the photosynthetic apparatus contrasts with the occurrence of esterified xanthophylls in the yellow chromoplasts of fruits and flowers.

Among the algae, the xanthophylls have been found to vary in three ways. In a given species, the proportions of the several pigments may alter in response to changes in the environment. In different plant classes, the xanthophylls differ in kind as well as in quantity. In some plant groups spatially unstable xanthophylls occur along with the stable or *trans* xanthophylls.

Of all the pigments now considered to comprise a part of the photosynthetic apparatus of autotrophic plants, the xanthophylls vary most. Except for a few specialized, pigmented bacteria, autotrophic plants contain chlorophyll *a* and *β*-carotene as the principal representatives of these two ubiquitous types of plant pigments. By contrast, several groups, such as the diatoms, dinoflagellates, yellow-green algae and higher plants, do not contain a single xanthophyll in common (Strain and Manning, 1943b).

Because of the abundance and wide distribution of diatoms and dinoflagellates, the principal xanthophylls of these organisms, fucoxanthin and peridinin, must comprise a major portion of the total amount of xanthophylls occurring in the world's vegetation. In the dinoflagellate, the proportion of peridinin relative to the two chlorophylls was quite large. On account of the strong absorption of light by peridinin in the spectral region where absorption by the chlorophylls is weak, studies of the photosynthetic activity of dinoflagellates in light of various wavelengths might yield information regarding the role of this unique xanthophyll in photosynthesis.

Interpretation of the photochemical activity of the individual leaf pigments depends upon knowledge of the physical state and spectral properties of the several pigments in the leaf itself. Spectral properties of the pigments determined in any one solvent or in colloidal suspension may not yield results precisely applicable to calculation of the absorption by each pigment in the living organism.

The variation in pigments between members of different algal classes, together with the constancy of the pigments in different members of a single class, provides a promising approach to the problem of phylogenetic relationship between the various classes of algae. For example, brown algae contain at least one xanthophyll, violaxanthin, common to the higher green plants, whereas diatoms and dinoflagellates contain none. Tribonema, sometimes considered closely related to the diatoms, contains none of the principal diatom xanthophylls, nor chlorophyll *c*. None of the algae investigated contain lutein, the common xanthophyll of green algae and of higher plants. These and other phylogenetic aspects of our observations on the chloroplast pigments of plants will be discussed elsewhere.

Adsorption phenomena. Reversal of the relative positions of adsorbed substances with changes in solvents and adsorbents indicates disproportionate variations in the adsorbability. It follows that the relative adsorbability of chemical substances is not determined by chemical structure alone; hence, great care must be exercised if deductions of molecular structure are to be based upon relative adsorbability (Strain, 1942a; 1942b).

Variations of the relative adsorbabilities of chemical compounds with changes in adsorbents and solvents suggest precautions to be employed in the use of the chromatographic adsorption method. For instance, if one solvent causes a given

pair of substances to be adsorbed in one sequence and if another solvent causes the same substances to be adsorbed in the inverse order, then there should be at least one mixture of the two solvents that will not effect a separation of the pair of adsorbed substances. Tests of the selectivity of a given adsorbent for separation of a mixture of given compounds can not, therefore, be regarded as conclusive unless different solvents and various mixtures of these solvents are used to develop the chromatograms. Determination of the homogeneity of chemical substances and the comparison of substances suspected of being identical through use of chromatographic adsorption methods (Strain, 1942b) will be most effective when various adsorbents and solvents are employed. Reversal of relative adsorbability with changes in the solvents suggests that the selectivity of the adsorbent may be just as dependent upon the nature of the solvents employed as upon the inherent properties of the adsorbent itself. Unless special precautions are taken, these changes in relative adsorbability increase the chances for confusion and error when substances are identified by the so-called mixed chromatogram (Strain, 1942b).

Most of the plant pigments that have been separated upon adsorption columns have yielded bands or zones with the highest concentration of pigment in the leading portions and with diminishing concentrations in the trailing portions (Strain, 1942a). The inverse behavior of adsorbed diadinoxanthin is difficult to interpret in relation to the theories regarding the distribution of an adsorbed pigment upon the columns (Strain, 1942a; DeVault, 1943).

The great color change observed upon adsorption of fucoxanthin and peridinin, in contrast to the slight change observed upon adsorption of neoxanthin, may result from interaction between the adsorbent and the pigments rather than from increased concentration of the xanthophylls at the interface. This effect may be analogous to that produced by addition of polar solvents to solutions of peridinin in nonpolar solvents (Figs. 3 and 6). It emphasizes the desirability of additional knowledge concerning the state of the pigments within the leaf, especially for interpretation of the photosynthetic reactions in various spectral regions (Dutton and Manning, 1941) and for interpretation of color changes that occur when algae are killed. The strong adsorption of some of the xanthophylls at the interface between water and petroleum ether may be utilized for separation of these pigments from other less adsorped constituents in the extracts of plants (Strain, 1943).

Summary

Carotenoid pigments of several groups of algae have been obtained through utilization of the chromatographic adsorption method. The selectivity of this method and the relative positions of the pigments in the columns have been found to vary with the solvents and adsorbents that were employed.

Xanthophylls of algae represent a large proportion of the carotenoid pigments produced in the world's vegetation. Most of the algal xanthophylls were readily convertible, reversibly, into one or more isomers that were separated on the adsorption columns. The principal xanthophylls were the more stable, presumably *trans,* isomers. Some of the labile isomers also appeared to be normal constituents of the cells. All the algal xanthophylls were unesterified.

The following xanthophylls have been obtained from each of six species of diatoms: diatoxanthin, diadinoxanthin (both new xanthophylls), fucoxanthin, neo-

fucoxanthin A, and neofucoxanthin B. From some eight species of brown algae there were obtained: diatoxanthin (occasionally, in traces), diadinoxanthin (occasionally, in traces), violaxanthin, a flavoxanthin-like xanthophyll, fucoxanthin, neofucoxanthin A, neofucoxanthin B and traces of other xanthophylls. The dinoflagellate *Peridinium cinctum* and an alga inhabiting a sea-anemone yielded the following xanthophylls: diadinoxanthin, dinoxanthin (a new xanthophyll), neodiadinoxanthin (a new xanthophyll), neodinoxanthin (a new xanthophyll), peridinin, and two or three isomers of peridinin. A yellow-green alga, *Tribonema bombycinum*, did not contain fucoxanthin, peridinin or chlorophyll *c*.

None of the algae examined contained lutein. All the algae examined contained β-carotene as the principal polyene hydrocarbon. In only one species, the diatom *Navicula torquatum*, were appreciable quantities of another carotene ϵ-carotene, observed.

A new xanthophyll, tareoxanthin, was obtained from flowers of the dandelion. A flavoxanthin-like xanthophyll from these flowers may not be identical with flavoxanthin from other sources. Violeoxanthin, a new xanthophyll, was isolated from the flowers of the pansy. Improved methods for the isolation of neoxanthin and violaxanthin from leaves are described. The violaxanthin b of leaves and violaxanthin of pansies are chromatographically identical.

Several groups of xanthophylls with almost identical characteristic spectral absorption curves have now been found. Neoxanthin, tareoxanthin and violeoxanthin fall into one group; violaxanthin, taraxanthin, and dinoxanthin comprise another group; and there are indications of several flavoxanthin-like pigments.

The solvent has a pronounced effect on the shape of the spectral absorption curves of peridinin and of fucoxanthin. In addition to indicating an effect of the solvent on the structure of the pigment molecule, this phenomenon also emphasizes the importance of knowledge concerning the spectral properties of the xanthophylls in the living plant, especially if these spectral characteristics are to be employed in an analysis of the photochemical activity of the plant pigments.

Of the pigments comprising the photosynthetic apparatus, the xanthophylls are subject to the greatest variation. Nearly two dozen of these pigments have been found in the green parts of various plants.

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